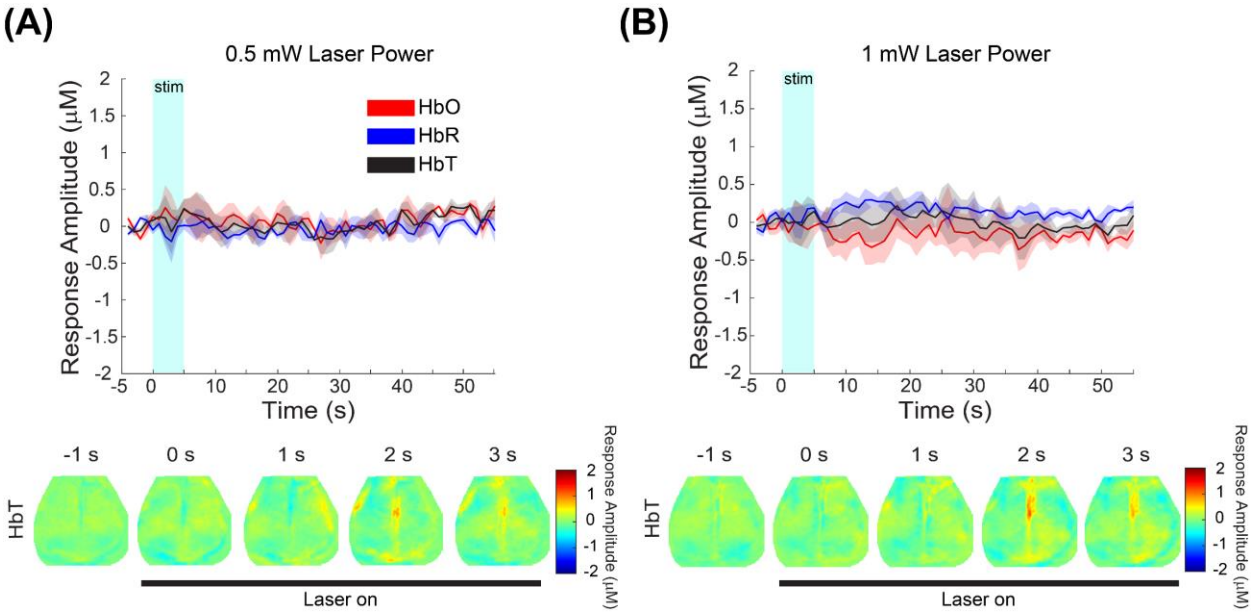
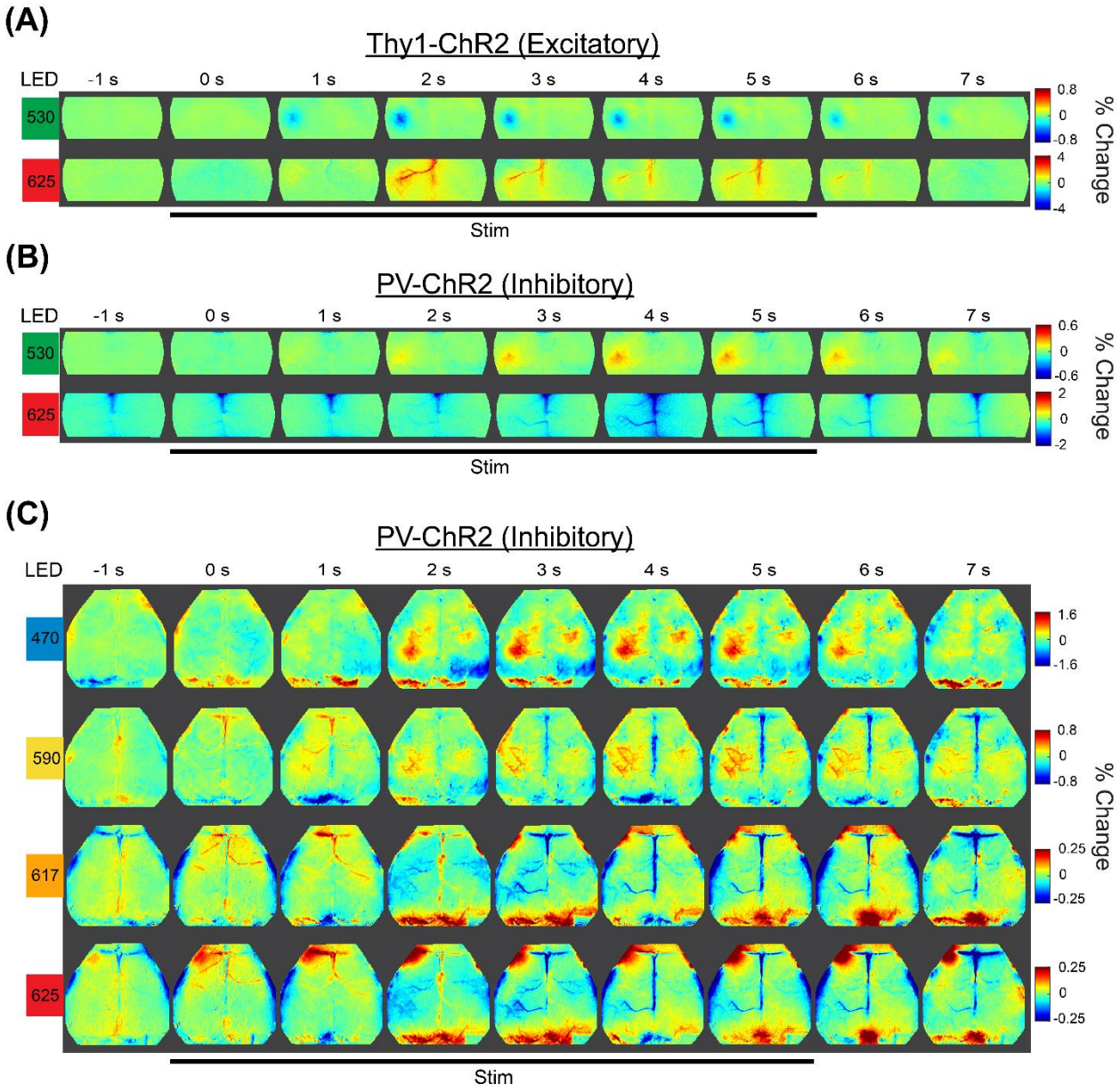
Supplementary methods

None

Supplementary figures



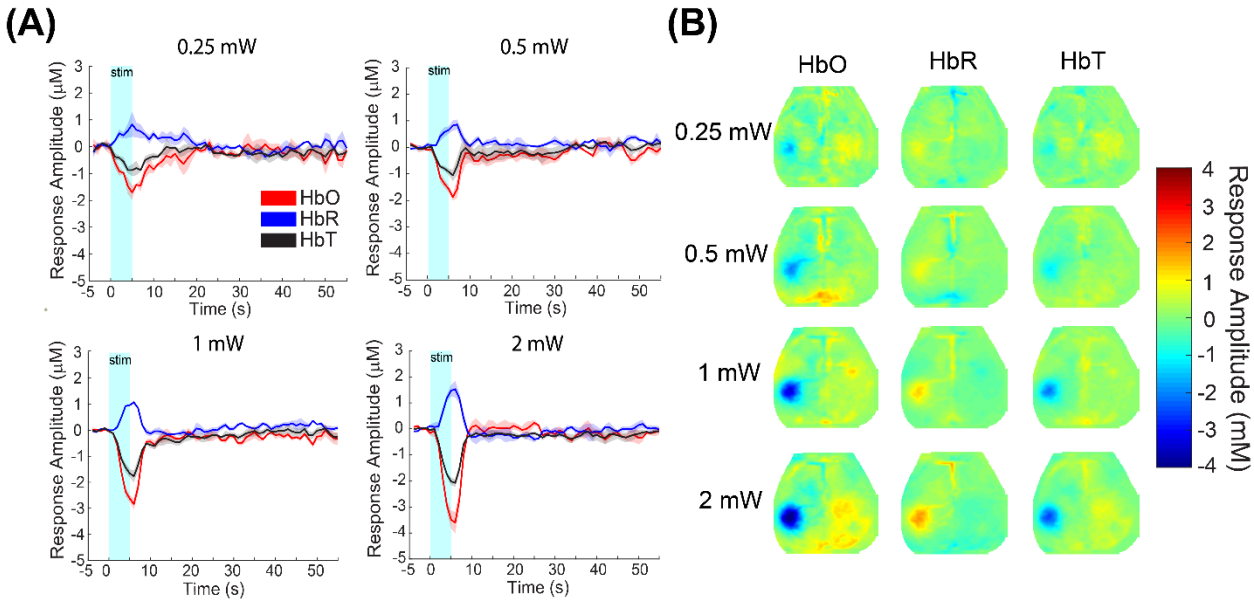
SUPPLEMENTARY FIGURE 1. Photostimulating wild-type (C57) mice does not induce stimulus-evoked hemodynamic activity.
A) Δ[HbO], Δ[HbR], and Δ[HbT] time traces generated during delivery of 0.5 mW photostimulus to C57 mice (n = 3). 473 nm photostimuli were delivered at 20 Hz as 5 ms pulses. Time-traces were calculated by averaging all pixels within a 3 pixel radius of the photostimulus site. No notable stimulus-evoked activity was observed during the stimulation period. Images of Δ[HbT] during the stimulation period reveal no notable stimulus-evoked activity at the site of stimulation (left barrel cortex).
B) Same as panel A) but using a 1 mW photostimulus.



SUPPLEMENTARY FIGURE 2. Raw-subtraction maps for PV-ChR2 and Thy1-ChR2 mice
A) Reflectance images for a representative Thy1-ChR2 mouse imaged with the Opto-OIS-LSCI system. Maps are shown for each LED used (530 nm and 625 nm) with no spatial binning (19.5 micron sized pixels). 473 nm photostimuli were delivered at 10 Hz as 5 ms pulses. Highly localized increases in 625 nm reflectance and decreases 530 nm reflectance suggest increases in [HbT] and [HbO] and decreases in [HbR]. Small-vessel changes were clearly visible during stimulation. The field of view for Opto-OIS-LSCI in panels A and B was reduced to accommodate a higher acquisition frame rate compared to Opto-OISI (panel C). More details can be found in Methods.
B) Same as panel A) except for a representative PV-ChR2 mouse during 20 Hz photostimulation. Decreases in 625 nm reflectance and increases in 530 nm reflectance suggest decreases in [HbT] and [HbO] and increases in [HbR]. Small-vessel changes were clearly visible during stimulation.

Photostimulation of PV-ChR2 mice elicited smaller magnitude changes than that of Thy1-ChR2 mice.

C) Raw-subtracted images for a representative PV-ChR2 mouse imaged with the Opto-OISI system. LEDs with wavelengths centered at 470 nm, 590 nm, 617 nm, and 625 nm were used with maps shown for each. As with B, 473 nm photostimuli was delivered at 20 Hz at 5 ms pulses. Maps of diffuse reflectance during photostimulation of barrel cortex are consistent with decreases in [HbT] and [HbO] and increases in [HbR]. For panel C only, global-signal regression was performed to remove global variance in light level fluctuations.



SUPPLEMENTARY FIGURE 3. Titrating photostimulus power in PV-ChR2 mice.

A) Δ[HbO], Δ[HbR], and Δ[HbT] time traces across photostimulus power levels (0.25 mW, 0.5 mW, 1 mW, and 2 mW) in 3 mice. Other photostimulus parameters were kept consistent (473 nm light, 20 Hz delivery, 5 ms pulses). Averaged time courses were determined by thresholding each mouse's peak Δ[HbT] map at 50% of max and averaging all time courses in pixels above that threshold. Time-traces exhibited higher SNR with higher photostimulus power (number of traces with SNR > 1.5: 44% for 0.25 mW data, 67% for 0.5 mW data, 89% for 1 mW data, and 98% for 2 mW data). A direct relationship existed between photostimulus power and peak response magnitude (further elaborated on in Fig. 3).

B) Δ[HbO], Δ[HbR], and Δ[HbT] peak maps across titrated power levels. A highly localized response in left barrel cortex was observed for all stimulus conditions and hemodynamic contrasts.